

A Clean Set of Claims

5. (Twice amended) A plasmid for expression of recombinant eucaryotic genes comprising:

a first transcription unit comprising a first transcriptional control sequence transcriptionally linked with a first 5'-untranslated region comprising a first synthetic intron, a first coding sequence, and a first 3'-untranslated region/poly (A) signal, wherein said first synthetic intron is between said first transcriptional control sequence and said first coding sequence; and

a second transcription unit comprising a second transcriptional control sequence transcriptionally linked with a second 5'-untranslated region comprising a second synthetic intron, a second coding sequence, and a second 3'-untranslated region/poly (A) signal, wherein said second synthetic intron is between said second transcriptional control sequence and said second coding sequence;

wherein the first and second synthetic introns both comprise 5' splice sites having a sequence CAGGTAAGT, and/or branch points having a sequence TACTAAC, and/or 3' splice sites having a sequence TTCTTTTTTCTCTTCACAGG.

10. (Twice amended) A plasmid for expression of recombinant eucaryotic genes, comprising an intron having variable splicing, a first coding sequence, and a second coding sequence, wherein the plasmid comprises:

a transcriptional control sequence transcriptionally linked with a first coding sequence and a second coding sequence;

a 5'-untranslated region;

an intron 5' to said first coding sequence;
an alternative 3' splice site located between the first and second coding sequences; and
a 3'-untranslated region/poly(A) signal,
wherein the intron comprises a 5' splice site having a sequence CAGGTAAGT, and/or a branch point having a sequence TACTAAC, and/or a 3' splice site having a sequence TTCTTTTTTTCTCTTCACAGG.

14. (Twice amended) A plasmid for expression of recombinant eucaryotic genes comprising:

a transcriptional control sequence transcriptionally linked with a first coding sequence, an IRES sequence, a second coding sequence, and a 3'-untranslated region/poly(A) signal, wherein said IRES sequence is between said first coding sequence and said second coding sequence; and

a synthetic intron between said transcriptional control sequence and said first coding sequence,

wherein the synthetic intron comprises a 5' splice site having a sequence CAGGTAAGT, and/or a branch point having a sequence TACTAAC, and/or a 3' splice sites having a sequence TTCTTTTTTTCTCTTCACAGG.

50. (New) The plasmid of claim 5 wherein the first and second synthetic introns both comprise 5' splice sites having the sequence CAGGTAAGT and branch points having the sequence TACTAAC and 3' splice sites having the sequence TTCTTTTTTTCTCTTCACAGG.

51. (New) The plasmid of claim 10 wherein the first and second synthetic introns both comprise 5' splice sites having the sequence CAGGTAAGT and branch points having the sequence TACTAAC and 3' splice sites having the sequence TTCTTTTTTTCTCTTCACAGG.

52. (New) The plasmid of claim 10 wherein either the 3' splice site or the alternative 3' splice site is weakened with respect to the other.

53. (New) The plasmid of claim 10 wherein either the 3' splice site or the alternative 3' splice site is weakened with respect to the other by changing three consecutive T's to A's.

54. (New) The plasmid of claim 10 wherein the 3' splice site has a sequence TTCTTTAAATCTCTTCACAGG and the alternative 3' splice site has a sequence TTCTTTTTTTCTCTTCACAGG.

55. (New) The plasmid of claim 14 wherein the synthetic intron comprise the 5' splice site having the sequence CAGGTAAGT, the branch point having the sequence TACTAAC, and the 3' splice site having the sequence TTCTTTTTTTCTCTTCACAGG.

56. (New) A synthetic transcription unit for efficient and accurate expression of recombinant eucaryotic genes, the transcription unit comprising a synthetic intron comprising:

a 5' splice site having a sequence MAGGTRAGT, wherein M = C or A and R = G or A;

a branch point having a sequence YNYTRAY, wherein Y = C or T, R = A or G and N = any base; and

a 3' splice site having a sequence Y₁₆NYAGG, wherein Y = C or T, and N = any base.

57. (New) The synthetic transcription unit of claim 56, wherein the 3' splice site has a sequence Y₃TTTTTTTY₆NYAGG, wherein Y = C or T, and N = any base.

58. (New) The synthetic transcription unit of claim 57, wherein the 3' splice site has a sequence TTCTTTTTTCTCTTCNYAGG, wherein Y = C or T, and N = any base.

59. (New) The synthetic transcription unit of claim 58, wherein the branch point and 3' splice site together have a sequence YNYTRAYGGY₁₆NYAGG, wherein Y = C or T, R = G or A, and N = any base.

60. (New) The synthetic transcription unit of claim 59, wherein the GG between Y and Y₁₆ of sequence YNYTRAYGGY₁₆NYAGG are substituted with from 2 up to 14 nucleotides of any base.

61. (New) The synthetic transcription unit of claim 59, wherein the branch point and 3' splice site together have a sequence YNYTRAYGGTTCTTTTTTCTCTTCNYAGG, wherein Y = C or T, R = G or A, and N = any base.

62. (New) The synthetic transcription unit of claim 61, wherein the branch point and 3' splice site together have a sequence TACTAACGGTTCTTTTTTCTCTTCACAGG.

63. (New) The synthetic transcription unit of claim 56, wherein the synthetic intron has a sequence CAGGTAAGT -N_x- TACTAACGGTTCTTTTTTCTCTTCACAGG, wherein x = 55-165.

64. (New) The synthetic transcription unit of claim 63, wherein the synthetic intron has a sequence CAGGTAAGTGTCTTC -N₇₇- TACTAACGGTTCTTTTTTCTCTTCACAGG.